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Role of Ca^{2+} in Prostaglandin E_2-Induced T-Lymphocyte Proliferative Suppression in Sepsis

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Prostaglandin E_2 (PGE_2) has been known to modulate immune responses by inhibiting T-cell activation following hemorrhagic and traumatic injury. Recently, we documented a sepsis-related depression in concanavalin A (ConA)-induced T-cell proliferation and intracellular Ca^{2+} (Ca^{2+}_i) mobilization. The present study evaluated the potential role of PGE_2 in the sepsis-related attenuation in Ca^{2+} signaling and proliferation in T cells. Sepsis was induced in rats by implanting into their abdomen fecal pellets containing *Escherichia coli* (150 CFU) and *Bacteroides fragilis* (10^4 CFU). A group of rats implanted with septic pellets were treated with indomethacin at three consecutive time points. Levels of PGE_2 in blood were measured with a radioimmunoassay kit. ConA-induced $[Ca^{2+}]_i$ mobilization in T cells obtained from indomethacin-treated and untreated rats was measured with Fura-2 and microfluorometry. We observed a 10-fold increase in PGE_2 levels in the circulation of septic rats compared with levels in rats implanted with bacterium-free sterilized pellets. The proliferative response and Ca^{2+}_i mobilization were significantly depressed in T cells obtained from septic rats 48 h after implantations compared with those in rats implanted with sterile pellets. However, treatment of rats with the cyclooxygenase inhibitor indomethacin prevented the sepsis-related depression in ConA-induced T-cell Ca^{2+}_i mobilization as well as proliferation. Further, incubation of T cells from nonimplanted control rats with PGE_2 resulted in a substantial depression in both T-cell proliferation and Ca^{2+}_i mobilization. The restoration of T-cell proliferation and Ca^{2+} signaling after indomethacin treatment of septic rats and the depression in the mitogen responsiveness in T cells previously exposed to PGE_2 suggest that the PGE_2 does play a significant role in the modulation of T-cell responses in septic rats and that such PGE_2-induced suppression in T-cell activation is likely due to an attenuation in Ca^{2+} signaling.

Prostaglandin E_2 (PGE_2) has been implicated in trauma- and burn injury-associated decreases in T-cell proliferation (20), interleukin-2 (IL-2) production (17, 20), and IL-2R expression (40) and a shift in T-cell subpopulation (4, 17). The impairment in T-cell functions could result in a decreased host resistance and increased susceptibility to the infection. Also, in vitro studies have shown PGE_2 to decrease T-cell proliferation (19, 31) and IL-2 production (3). Under conditions such as trauma, sepsis, and shock, the inflammatory response is accompanied by an increased production of PGE_2 by monocytes (17) and elevated levels of PGE_2 in blood (5, 34). Despite extensive investigation in the last two decades, there has been a controversy regarding not only the mechanisms involved in PGE_2-mediated suppression of T cells but also the role of PGE_2 in trauma, sepsis, and shock. A number of in vitro studies have correlated an elevation in intracellular cyclic AMP (cAMP) with an inhibition of T-cell function (31), but a definitive cellular mechanism of PGE_2-mediated suppression in T-cell functions has remained unknown. Previous studies from our laboratory showed the involvement of a disturbed intracellular Ca^{2+} (Ca^{2+}_i) homeostasis in sepsis-related inhibition of T-cell activation (12). An elevation in $[Ca^{2+}]_i$ is an immediate event in T-lymphocyte activation following the stimulation of T-cell receptor with antigen, mitogen, or antibodies to the T-cell receptor-CD3 complex (2, 41). The present study evaluated the potential role of PGE_2 in the modulation of Ca^{2+} signaling in T cells leading to the suppressed proliferation of these cells in sepsis. We assessed the effects of PGE_2 synthesis blockade by indomethacin on concanavalin A (ConA)-induced T-cell $[Ca^{2+}]_i$ elevation. Previous studies by other investigators evaluated the efficacy of indomethacin treatment of thermally injured animals in restoring T-cell proliferation and IL-2 production (16, 17, 30).

MATERIALS AND METHODS

Animal model of sepsis. Male Sprague-Dawley rats (200 to 225 g) obtained from Harlan (Harlan Sprague-Dawley, Inc., Indianapolis, Ind.) were used. Sepsis was induced by implanting 1 cm^3 of presterilized fecal pellets impregnated with *Escherichia coli* ATCC 25992 (150 CFU) and *Bacteroides fragilis* (10^4 CFU) into the rat abdomen. Rats implanted with sterilized pellets without the bacteria are referred to as sterile. A febrile response (>38°C), decreased sensibilities to touch, diarrhea, eye hemorrhage, and piloerection were characteristic findings in septic rats on days 1 to 3 postimplantation. The sterile rats exhibited the febrile response without a marked appearance of other signs of sepsis on day 1 after implantation. Whereas no mortality occurred in the sterile rats, approximately 45% of all septic rats died between days 1 and 2 after implantation. Details of this model of gram-negative intra-abdominal sepsis have been reported elsewhere (1, 8).

Treatment of animals with indomethacin. Indomethacin (2 mg/kg) was administered intraperitoneally initially 2 h prior to implantation and 24 and 36 h after implantation.

Measurements of plasma levels of PGE_2. Blood was drawn via cardiac puncture into a heparinized tube from control, sterile, and septic rats 40 to 48 h after implantation. The serum was separated immediately by centrifugation, aliquoted, and stored at −70°C until use. Indomethacin (10 μg/ml) was added to the samples to block the de novo synthesis of prostaglandins. The measurements of PGE_2 contents in the serum sample were carried out with a radioimmunoassay kit (Amersham). The sensitivity of the assay was in the range of 2 to 1,600 pg/ml.

T-cell preparation. Rats were sacrificed, and their spleens were removed aseptically and processed to obtain T cells, as previously described (12, 26). Briefly, spleens were gently ground to prepare single-cell suspensions, which were then subjected to density gradient centrifugation with Ficoll-Paque (Pharmacia, Sweden) to remove erythrocytes. Splenocytes appearing at the interphase of Ficoll and the medium were collected. To obtain pure T cells, splenocytes

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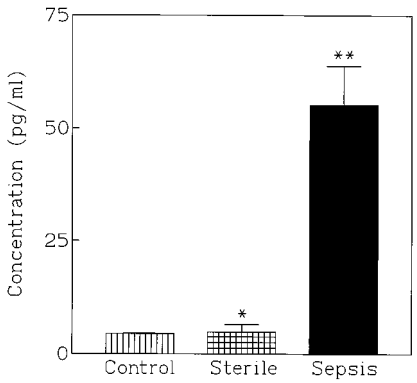

FIG. 1. Plasma levels of PGE_2 in nonimplanted control rats and in sterile and septic rats 48 h after implantation. Values are means (± standard errors) for five animals. *, $P > 0.05$, sterile versus nonimplanted control group; **, $P < 0.01$, sepsis versus sterile and nonimplanted control groups.

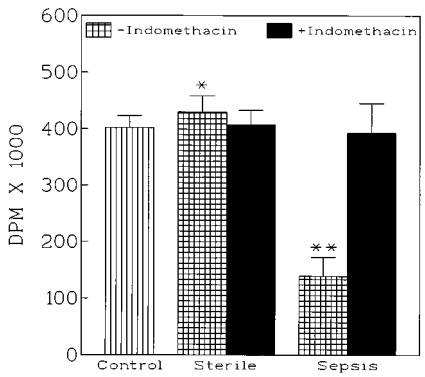

FIG. 2. ConA-induced proliferative responses of T cells from nonimplanted control rats and from sterile and septic rats 48 h after implantation. Values are means (± standard errors) for six or more animals. *, $P > 0.05$, sterile versus nonimplanted control or sterile + indomethacin group; **, $P < 0.01$, sepsis versus control, sterile, or sepsis + indomethacin group.

were passed through a nylon wool column preequilibrated with Hanks balanced salt solution supplemented with 10 mM HEPES (*N*-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), 5% fetal calf serum, and 50 μg of gentamicin per ml. After 45 to 60 min of incubation of columns containing cells at 37°C, T cells were obtained by eluting the columns with 30 to 40 ml of warm Hanks balanced salt solution at a flow rate 1 drop/s.

Determination of T-cell proliferation. T cells were resuspended in RPMI 1640 (GIBCO) supplemented with L-glutamine (2 mM), 2-mercaptoethanol (50 μM), HEPES (10 mM), gentamicin (50 μg/ml), and fetal calf serum (10%) and cultured at a density of 5×10^5 cells per well in a 96-well plate at 37°C with 5% CO_2 in the presence or absence of ConA (5 μg/ml; Sigma Chemical Co., St. Louis, Mo.). After about 66 h of culture, a trace quantity of [^{3}H]thymidine was added to each well, and the cells were cultured for an additional 6 h. Cells were harvested on glass fiber filter strips, using a 24-well PHD cell harvester (Cambridge Technology, Cambridge, Mass.), and thymidine activity was determined in a liquid scintillation counter. The proliferative response was expressed as radioactivity (disintegrations per minute) incorporated into the cells.

Measurements of T-cell $[Ca^{2+}]_i$. The measurements of T-cell $[Ca^{2+}]_i$ was performed following the methods reported earlier (12). Briefly, nylon wool-purified T cells were resuspended at a density of 5×10^7 cells per ml and loaded with 2 μM Fura-2/AM (Molecular Probes, Inc.) for 1 h at room temperature. The unbound Fura-2 was removed by washing the cells with Hanks balanced salt solution. The Fura-2-loaded cells in suspension were transferred to a fluorometer cuvette maintained at 37°C. Fluorescence signals were recorded with a Hitachi spectrofluorophotometer (model F-2000) at excitation wavelengths of 340 and 380 nm and an emission wavelength of 510 nm. The cells were stimulated with ConA (50 μg/ml) after 40 to 50 s of the initial response (basal). A peak elevation in $[Ca^{2+}]_i$ usually occurred at 100 to 120 s after the addition of ConA. This peak $[Ca^{2+}]_i$ was taken as the ConA-induced elevation. Triton X-100 (0.2%) was added to the cells to obtain maximum fluorescence change, and finally the minimum fluorescence change was recorded after the addition of EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; 10 mM]. The fluorescence changes due to ConA over the basal response were converted to changes in $Ca^{2+}{}_i$ concentration by using the following equation (21):

$$[Ca^{2+}] = k_d \frac{R - R_{min}}{R_{max} - R} \times \frac{S_{f380}}{S_{b380}}$$

where k_d = 224 nM; R = ratio of fluorescence (F), i.e., F_{340}/F_{380} under basal or ConA-stimulated conditions; $R_{max} = F_{340}/F_{380}$ ratio after the addition of Triton X-100; $R_{min} = F_{340}/F_{380}$ ratio after addition of EGTA; S_{f380} = fluorescence signal at 380 nm due to free Fura-2; and S_{b380} = fluorescence signal at 380 nm due to free Fura-2 bound to Ca^{2+}.

RESULTS

Plasma levels of PGE_2. Figure 1 shows plasma levels of PGE_2 in septic and sterile rats 40 to 48 h after implantations. The plasma levels of PGE_2 in control rats were 4.45 ± 0.05 pg/ml, which were not significantly different from that observed in sterile rats (4.8 ± 1.65 pg/ml). However, the plasma levels of PGE_2 measured in the septic rats were significantly elevated (55.2 ± 8.65; $P < 0.01$) compared with those of sterile and nonimplanted control rats.

T-cell proliferation. To explore the possible role of PGE_2 in sepsis-induced suppression of T-cell proliferation, we treated the septic as well as sterile rats with indomethacin (2 mg/kg) at three consecutive time points. As shown in Fig. 2, the proliferative response in the cells from control rats (401,884 ± 21,333 dpm) was not significantly different from that observed in the T cells obtained from sterile rats 48 h after implantations (429,810 ± 28,306 dpm). However, ConA-induced proliferative capacity in T cells obtained from septic rats was significantly lower (138,618 ± 33,568 dpm; $P < 0.01$) than that observed in sterile rats 48 h after implantations. This indicated a 68% inhibition in the proliferation of T cells from septic rats compared with that of the sterile rats.

As shown in Fig. 2, the administration of indomethacin to sterile rats did not modify T-cell responsiveness to ConA. The ConA-induced proliferation in T cells obtained from septic rats after indomethacin treatment was found to be 392,186 ± 52,746 dpm, which was not significantly different from the T-cell proliferative responses observed in sterile and control rats ($P > 0.05$). This suggested that indomethacin treatment of septic rats significantly restored the mitogen responsiveness of T cells compared with that observed in T cells from untreated septic rats ($P < 0.01$). Indomethacin treatment of rats did not significantly improve the survivability of septic rats.

T-cell $[Ca^{2+}]_i$ measurements. The measurements of basal and ConA-mediated intracellular Ca^{2+} mobilization in T cells obtained from sterile and septic rats before and after indo-

TABLE 1. Intracellular $[Ca^{2+}]$ before and after ConA stimulation in T cells obtained from nonimplanted control rats and from sterile and septic rats 48 h after implantations

Experimental group	Intracellular $[Ca^{2+}]$ (nM)[a]	
	−ConA (basal)	+ConA (stimulated)
Control	122.29 ± 5.03	220.0 ± 7.58
Sterile	113.0 ± 5.35	197.4 ± 10.62[b]
Sterile + indomethacin	104.8 ± 1.67	185.3 ± 6.43
Sepsis	106.0 ± 3.31	148.6 ± 4.38[c]
Sepsis + indomethacin	116.3 ± 3.60	191.8 ± 10.95

[a] Values are means ± standard errors for six or more animals.
[b] $P > 0.05$, sterile versus nonimplanted control or sterile + indomethacin group.
[c] $P < 0.01$, sepsis versus nonimplanted control, sterile, or sepsis + indomethacin group.

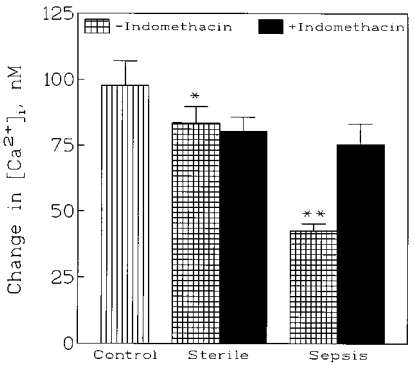

FIG. 3. ConA-induced mobilization in intracellular Ca^{2+} in T cells from nonimplanted control rats and from sterile and septic rats 48 h after implantation. Values are means (± standard errors) for six or more animals. *, $P > 0.05$, sterile versus nonimplanted control or sterile + indomethacin group; **, $P < 0.01$, sepsis versus control, sterile, or sepsis + indomethacin groups.

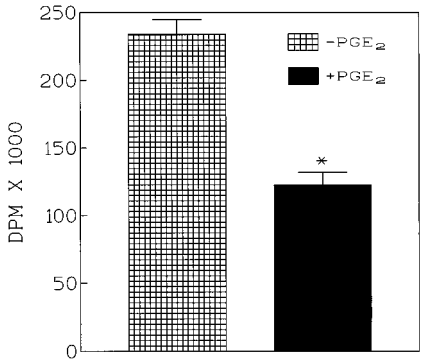

FIG. 4. PGE_2-mediated suppression of ConA-induced proliferation of T cells from nonimplanted control rats. Values are means (± standard errors) for six animals. *, $P < 0.01$, T cells incubated with PGE_2 versus T cells without PGE_2.

methacin treatment are shown in Table 1. In the present study, the basal $[Ca^{2+}]_i$ in unstimulated T cells from nonimplanted control rats was 122.29 (± 5.03) nM. This was not significantly different from the basal $[Ca^{2+}]_i$ values in T cells obtained from sterile and septic rats. The addition of ConA to the T cells from control rats resulted in significant elevations in $[Ca^{2+}]_i$ (220.0 ± 7.58 nM). The ConA-induced elevations in the $[Ca^{2+}]_i$ in T cells obtained from sterile rats were not significantly different from those from control rats. However, ConA-induced elevations in $[Ca^{2+}]_i$ in T cells obtained from septic rats (148.6 ± 4.38 nM) were significantly less ($P < 0.01$) than those in cells from nonimplanted control and sterile rats. The differences between basal $[Ca^{2+}]_i$ and the elevated $[Ca^{2+}]_i$, referred to as the mobilized $[Ca^{2+}]_i$ in response to ConA, were calculated for T cells obtained from control rats and indomethacin-treated and untreated sterile and septic rats and are shown in Fig. 3. The indomethacin treatment did not affect the basal $[Ca^{2+}]_i$ or ConA-induced $[Ca^{2+}]_i$ mobilization in T cells from sterile rats (Fig. 3). Although there was no apparent change in the basal $[Ca^{2+}]_i$ in T cells obtained from indomethacin-treated and untreated septic rats, the ConA-induced $[Ca^{2+}]_i$ mobilization was higher in the T cells obtained from indomethacin-treated septic rats than in those from untreated septic rats. The restoration of T-cell $Ca^{2+}{}_i$ mobilization and proliferation after indomethacin treatment in septic rats suggests the involvement of PGE_2 in the depression of T-cell function.

Effect of PGE_2 on T-cell proliferation in vitro. To evaluate direct inhibitory effects of PGE_2 on ConA-induced T-cell proliferation, cells obtained from control rats were cultured with ConA in the presence and absence of PGE_2. As shown in Fig. 4, the proliferative response in T cells cultured without added PGE_2 (224,798 ± 10,632 dpm) was found to be significantly higher ($P < 0.01$) than that observed in the T cells incubated in the presence of PGE_2 (122,658 ± 9,246 dpm). This suggested a 45% inhibition in ConA-induced proliferation in T cells incubated with PGE_2 compared with cells cultured without PGE_2.

The proliferative response of ConA-stimulated T cells cultured without PGE_2 is lower (224,798 ± 10,632 dpm) than that observed in nonimplanted control rat T cells (401,884 ± 21,331 dpm) as well as sterile rat T cells (429,810 ± 28,306 dpm) (Fig. 2). This variability in control rat T-cell proliferation responses may be related to different batches of ConA employed in the two sets of experiments.

Effect of PGE_2 on T-cell $Ca^{2+}{}_i$ mobilization in vitro. In experiments on T-cell $Ca^{2+}{}_i$ mobilization in vitro, T cells from control rats were first cultured overnight and then exposed to PGE_2 (100 nM) for 2 h before being stimulated with ConA. Consistent with earlier observations, no change in the basal $[Ca^{2+}]_i$ was observed in T cells incubated with (117.5 ± 2.28 nM) and without (116.2 ± 1.72 nM) PGE_2. However, ConA-induced elevation in $[Ca^{2+}]_i$ was significantly higher in T cells cultured in media alone (325.91 ± 4.48 nM) compared with cells incubated with PGE_2 (218 ± 1.96 nM). The difference in the unstimulated and ConA-stimulated $[Ca^{2+}]_i$ was also significantly lower in the T cells incubated with PGE_2 ($P < 0.01$) compared with cells incubated in PGE_2-free media (Fig. 5). It should be noted that in these experiments the ConA-induced elevation in $[Ca^{2+}]_i$ in media alone (325.91 ± 4.48 nM) is substantially greater than that observed in T cells studied prior to the overnight culture (220 ± 7.58 nM; Table 1). The greater response to ConA in the cells cultured overnight could be due to an improved functional integrity in these cells compared with the freshly isolated cells without an overnight culture. Another result of note is that ConA caused the proliferative response in accompaniment with a 2.81-fold increase in $[Ca^{2+}]_i$ in control cells cultured overnight while it failed to support the proliferative response with an accompanying 1.8-fold increase in $[Ca^{2+}]_i$ in the PGE_2-exposed cells cultured overnight. This would mean that a mere doubling of $[Ca^{2+}]_i$ may not be an adequate $Ca^{2+}{}_i$ signal to lead to proliferation in the latter cells,

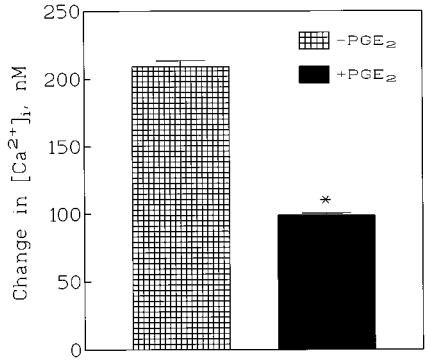

FIG. 5. PGE_2-mediated suppression of ConA-induced mobilization of Ca^{2+} in T cells. T cells obtained from nonimplanted control rats were incubated for 2 h with PGE_2 and then ConA-induced mobilization of $[Ca^{2+}]_i$ was measured. Values are means (± standard errors) for six animals. *, $P < 0.01$, T cells incubated with PGE_2 versus T cells without PGE_2.

a finding in some contrast to our other observations (12) (Table 1) with freshly prepared control T cells in which a doubling of $[Ca^{2+}]_i$ would appear to be adequate for a proliferative response. Overall, these observations suggest that there might be differences in the threshold of $[Ca^{2+}]_i$ elevations required for the elicitation of the proliferative response in the freshly isolated T cells versus control T cells cultured overnight. However, our studies do not provide any definitive information on either the minimum ConA-induced $[Ca^{2+}]_i$ elevation required for proliferation of the freshly isolated cells and cells cultured overnight or a change in the $[Ca^{2+}]_i$ elevation threshold with PGE_2 needed to potentially inhibit the proliferative responses.

DISCUSSION

The present study demonstrated significantly elevated levels of PGE_2 in the circulation in septic rats and a correlatable decrease in ConA-mediated T-cell proliferation and intracellular Ca^{2+} mobilization. Further, PGE_2 blockade with indomethacin administration to rats restored T-cell proliferation and $Ca^{2+}{}_i$ mobilization, and incubation of control rat T cells with PGE_2 suppressed ConA-mediated T-cell proliferation and $Ca^{2+}{}_i$ mobilization. The restoration of T-cell responses by blocking of PGE_2 in vivo and the suppression of T-cell responses after their exposure to PGE_2 in vitro support the idea that PGE_2 plays a role in the modulation of immune responses. Earlier studies had shown that an inflammatory response to major trauma or burn injury was accompanied by elevated levels of PGE_2 in blood (5, 15, 34) as well as increased production of PGE_2 by monocytes (17). Such elevated levels of PGE_2 were correlated with a decrease in IL-2 production and T-cell proliferation (16, 17).

Although a number of studies in the past have implicated an immunosuppressive role of PGE_2 during hemorrhagic and traumatic injury (9), the mechanisms of PGE_2-mediated suppression in T-cell functions are not yet understood. Faist et al. (18) showed that stimulation of T-cell blastogenesis was suppressed with mitogen alone, but mitogen plus phorbol myristate acetate led to normalization of both IL-2 mRNA expression and IL-2 secretion in trauma patients. This suggested that a defect in T-cell signaling occurred prior to protein kinase C (PKC) activation. Our previous observations showing a disturbance in Ca^{2+} homeostasis and its correlation with deranged T-cell proliferation in septic rats (12) are in keeping with these studies. Recently, Hoyt et al. (24) also documented an attenuation in $Ca^{2+}{}_i$ mobilization in T cells from patients with trauma compared with that in T cells from healthy volunteers. Interactions of a mitogen such as ConA or the monoclonal anti-CD3 antibody with the T-cell receptor-CD3 complex elicit a cascade of intracellular events, including the activation of phospholipase C gamma (33). As a result, inositol triphosphate and diacyl glycerol (DAG) are generated, leading to the release of Ca^{2+} from internal stores and the activation of PKC (2). In addition to the IP_3-mediated release of Ca^{2+} from internal stores, an influx of calcium through the plasma membrane channel is known to contribute to a prolonged elevation in $[Ca^{2+}]_i$ (25). This prolonged elevation in $[Ca^{2+}]_i$ also plays an essential role in T-cell activation and the subsequent proliferation of T cells (44). Our demonstration of a disturbed Ca^{2+} mobilization in sepsis is consistent with a defect preceding the PKC step because of a requirement of the Ca^{2+} signal for optimal PKC activation.

Many of the earlier studies have shown that PGE_2 interactions with T cells in vitro resulted in elevations of the cAMP level (38) and that such an elevated intracellular cAMP level in T cells mediated the proliferative disturbances (6, 29, 32). Although a precise role of Ca^{2+} signaling in PGE_2-mediated suppression of T-cell functions has remained controversial (11, 28, 29), our findings of suppressed $Ca^{2+}{}_i$ mobilization in T cells incubated with PGE_2 support the suggestion that PGE_2 directly affects Ca^{2+} signaling. Moreover, previously documented studies showing increased cAMP levels with correlatable decreases in phospholipase C gamma, inositol phosphate turnover (28), and inositol triphosphate generation (38) also implicate a role for Ca^{2+} in PGE_2-mediated T-cell suppression. The restoration of $Ca^{2+}{}_i$ signaling with a simultaneous reversal in T-cell proliferation in indomethacin-treated septic rats also indicates the mediation of PGE_2 effects through $Ca^{2+}{}_i$ signaling.

Kammer (27) presented evidence that the T-cell cAMP signal activated protein kinase A by dissociating it into two regulatory and two active subunits. This, in turn, presumably led to the inhibition of IL-2 expression, IL-2 production, and mitogen-induced proliferation. The cAMP-protein kinase A pathway is also known to negatively modify the phosphoinositide-PKC pathway, Ca^{2+} fluxes, and Ca^{2+}-calmodulin metabolism in T lymphocytes (7, 27, 35). Paliogianni et al. (35) documented that PGE_2 and other cAMP elevating agents can downregulate Ca^{2+}-calmodulin-dependent calcineurin-induced transcription of the IL-2 gene (13). The IL-2 enhancer region is known to have sites for at least six nuclear proteins that are produced either constitutively (NF-IL-2A and NF-IL-2B) or after stimulation of T cells (NF-AT, NF kB, AP-1, and CD28 RC) (14, 42). Thus, inhibition of IL-2 nuclear transcription by cAMP could result from either decreased production or interference with the binding or the function of transcription factors necessary for IL-2 transcription. Recently, Chen and Rothenberg (10) have shown decreased levels of NF kB and a newly described TGGGC binding factor in the nuclear extracts of T cells previously exposed to forskolin. Moreover, the reduction in NF kB and TGGGC was found to be mediated through cAMP-dependent activation of protein kinase A (10). These studies imply that PGE_2 may act at multiple steps to inhibit T-cell functions. Although we did not evaluate IL-2 transcription regulation, the interference with calcium mobilization, as observed in this study, would be expected to disturb IL-2 gene transcription.

PGE_2 also interferes with in vivo interactions between monocytes and T cells, leading to suppression of antigen presentation to T cells and inhibiting the monocyte expression of the class II major histocompatibility complex (39). This could imply that the defect in the antigen presentation by an accessory cell contributes to attenuated T-cell activation in vivo. The observed inhibition in the proliferation of T cells directly exposed to PGE_2 in the present study suggests that PGE_2 can directly affect proliferation. The direct effect of PGE_2 is also supported by previous studies (19, 31).

The activation of T cells is an essential component in the generation of an effective and competent immune response because the cytokines produced by these cells turn on other cell systems required for both humoral and cell-mediated immunity (36). The interdependence of T cells and macrophages in killing of gram-negative bacteria suggests that an abrogation in this coordinated defense mechanism may have detrimental effects. Thus, the depression in the T-cell activation because of an effect of PGE_2 on T cells and/or inadequate antigen presentation by macrophages and other antigen-presenting cells may contribute to a decrease in host resistance in sepsis. In the present study, we have not assessed immunosuppression per se; however, previous studies have demonstrated immune suppression following thermal injury by showing anergy to recall antigens (22, 43), delayed homograft rejection (37), and a

decreased response to T-cell mitogens (43). Although these studies have not shown a direct involvement of PGE_2 in post-burn-associated immunosuppression, the improvement in the host susceptibility to infection after a cyclooxygenase blockade (2, 23) supports a potential role for PGE_2 in such immunocompromised hosts.

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